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PP-049 **The novel role of downstream of kinase-1 in the ovalbumin-induced murine model of asthma**

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Background: The downstream of kinase (DOK)-1, a 62 kDa adaptor protein, negatively regulates the protein tyrosine kinase (PTK) pathway in mast cells, but the role of DOK-1 in a murine model of asthma has not been defined.
Methods: In this study, we have demonstrated a novel regulatory role of DOK-1 in airway inflammation and physiologic responses in a murine model of asthma using lentiviral vector containing DOK-1 cDNA or DOK-1-specific ShRNA. The OVA-induced inflammatory cells, airway hyperresponsiveness, Th2 cytokine expression, and mucus response were significantly reduced in DOK-1 overexpressing mice compared to OVA-challenged control mice.
Results: The transgenic introduction of DOK-1 significantly stimulates the activation and expression of STAT-4 and T-bet, while impressively inhibits the activation and expression of STAT-6 and GATA-3 in the airway epithelial cells. On the other hand, DOK-1 knockdown mice enhanced STAT-6 expression and its nuclear translocation compared to OVA-challenged control mice.
Conclusions: When viewed in combination, our studies demonstrate DOK-1 regulates allergen-induced Th2 immune responses by selective stimulation and inhibition of STAT-4 and STAT-6 signaling pathways, respectively. These studies provide a novel insight on the regulatory role of DOK-1 in allergen-induced Th2 inflammation and airway responses that has therapeutic potential for asthma and other allergic diseases.

PP-050 **Effect of resveratrol on IFN-gamma-induced expression of indoleamine 2,3-dioxygenase and tryptophanyl tRNA synthetase in murine bone marrow-derived dendritic cells**

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Background: Indoleamine 2,3-dioxygenase (IDO) is an essential enzyme that degrades the essential amino acid tryptophan. In local micro environment, excessive IDO expression is causing tryptophan depletion, result in suppression of effector T cell function and induction of T cell tolerance. Whereas, Tryptophanyl-tRNA-synthetase (TTS) is a constitutively expressed cytoplasmic enzyme that mediates the association of tryptophan with its specific tRNA. The complex of tryptophan with its tRNA, as a reservoir of tryptophan for protein synthesis, and then nullifies IDO-mediated deprivation of tryptophan. Resveratrol (trans-3,4′,5-trihydroxystilbene) is a natural polyphenol found in grapes and grape products. A number of studies have reported that resveratrol have a wide range of biological and pharmacological activities. However, effect of resveratrol on IDO and TTS expression is nonexistent.
Methods: Generation of BM-derived Murine DCs, Western blotting, Mixed Lymphocyte Reaction by using Transgenic ovalbumin (OVA) specific CD8+ T-cells.
Result: In this study, we found that resveratrol significantly inhibited IDO expression, but enhanced TTS expression in dendritic cells (DC). And we observed that IDO and TTS expression regulated by resveratrol is through a GSK-3 activity. Interestingly we observed that resveratrol (50 mg/kg) suppressed tumor growth in tumor-bearing mice. In this mice, IDO expression is inhibited but TTS expression was *changeless*.
Conclusion: These findings suggest that resveratrol regulates tumor-mediated immune tolerance and can be used for major adjuvant of anti-cancer therapy through the regulation of these two distinct enzymes.

PP-051 **Curcumin suppresses the induction of indoleamine 2,3-dioxygenase by blocking the JAK–PKC-δ–STAT1 signaling pathway in IFN-γ-stimulated murine dendritic cells**

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Indoleamine 2,3-dioxygenase (IDO) catalyzes the initial and rate-limiting step in the degradation of tryptophan, and is)-stimulated dendritic cells (DCs). IDO)strongly induced in interferon-γ (IFN-γ has recently been established as a key enzyme in T-cell suppression-mediated immune tolerance to tumors. STAT1 phosphorylation appears to play an important role in the control of IDO expression by IFN-γ, but the precise regulatory mechanism remains obscure. Here we present a novel mechanism of IFN-γ-induced IDO expression in bone marrow-derived dendritic cells (BMDCs).
In addition, we demonstrate that curcumin, an active component of turmeric, significantly inhibited the induction of IDO expression and activity by IFN-γ. We found that curcumin suppressed STAT1 activation by directly inhibiting JAK1/2 and PKC-δ phosphorylation in BMDCs, suppressing the subsequent translocation and binding of STAT1 to the GAS element of the IRF-1 promoter. Coincident with these inhibitory effects on IFN-γ-induced IDO expression, curcumin reversed IDO-mediated suppression of T-cell responses.
Our results thus suggest that down-regulation of IDO in DCs is an important immunomodulatory property of curcumin that may be exploited therapeutically in the control of cancers.

PP-052 **Difficulties in diagnosis of Visceral Leishmaniasis in India**

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Background: Visceral Leishmaniasis (VL) is a vector borne anthrozoonotic disease caused by a protozoan, *Leishmania donovani*, of *Trypanosomatidae* family. It is an endemic disease that covers 88 countries (16 developed and 72 developing) with a total of 350 million people at risk and 12 million cases of infection.
Methods: The most precise methods used for its diagnosis of parasites includes the analysis of spleen & liver smear (90%), Bone Marrow smear (80%), sternal or iliac crest puncture but these are not reliable and cumbersome/painful also. Various serological tests like indirect haemagglutination assay (IHA), countercurrent immuno-electrophoresis (CCIEP), Immunodiffusion (ID), Direct agglutination test (DAT), Indirect fluorescent antibody test (IFAT), ELISA etc. are used to diagnose VL. These tests are useful for both laboratories as well as for field and for the screening of large number of samples rapidly. The most sensitive and specific test is ELISA, since these tests are based on the antibody concentration hence are not suitable for immunocomprised (AIDS) patients. PCR based methods are also used to detect the VL patients by targeting 18S ribosomal gene.
Results: Recently, a latex agglutination test (KATEX) has been developed for the detection of leishmanial antigens in the urine of patients by monoclonal antibodies against VL

antigen in urine, which shows a sensitivity of 68–100% and specificity of 100% in beginning trials.
Conclusion: Our research is centralized to develop a detection method either from isolated specific antigen or from serum or any other fluid / tissues taken from kala-azar patients, which is easy to carry out and efficient for the diagnosis of visceral leishmaniais in field as well as in laboratory condition where lack of sophisticated instruments and expertise persons.

PP-053 Glypican-3 amino terminal marker for early detection of HCC

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Introduction: HCC is the 6th common cancer. Global increase of hepatitis B and C infection, the incidence of HCC has been steadily increasing. Egypt seroprevalence of HCV in Nile delta was 20–35%. AFP had limited sensitivity 60% and specificity 90% for small HCC. GPC-3 oncofetal protein over expressed in HCC.
Aim: Evaluating the validity of Glypican-3 as an early detector of HCC.
Material: 10 healthy controls and 40 HCV positive patients: 10 patients with chronic hepatitis C virus infection, 10 patients with compensated cirrhosis [Child–Pugh class A and B], 10 patients with decompensated cirrhosis [Child–Pugh class C], 10 patients with HCC.
Methods: Liver functions: ALT, AST, Bilirubin (T), Albumin, γGT. Tumor markers: AFP and GPC-3.Viral markers: HCV antibodies, HBs Ag and HBc Ab.
Results: The median value of GPC-3 in HCC, DC, CC was significantly higher than chronic hepatitis and control groups. No significant correlation found between AFP and GPC-3. AUROC of AFP was 0.85 & AUROC of GPC-3 was 0.84. The diagnostic sensitivity of AFP (20 ng/ml) was 70% with PPV 53.8%. The specificity was 85% with NPV 91.9%. While the diagnostic Sensitivity of GPC-3 (2 ng/ml) was 100% with PPV 27%. The specificity was 42.2% with NPV 100%. Combined serial approach of AFP and GPC-3 improved the specificity to 87.5%.
Conclusion: GPC-3 although it is a serological test for early detection of HCC, it showed limited specificity, where It is detected in different stages of chronic liver disease, as it is an oncofetal protein produced by regenerating liver cells. The diagnostic signature approach for simultaneous determination of AFP and GPC-3 may improve the prediction accuracy of HCC patients in those showing seronegativity to AFP.

PP-054 Study of portal and systemic levels of nitric oxide, endothelin-1 and procollagen III peptide in chronic liver disease in Egypt

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Egypt has one of the highest incidence of liver diseases in the world with prevalence of schistosomiasis. NO diffuses into cytosol of adjacent vascular smooth muscle cells play a role in the pathogenesis of vasodilation. Endothelial cells also produce the most potent vasoconstrictor agent endothelin (ET-1).
Aim: Evaluation of nitric oxide and endothelin-1 and procollagen III peptide in patients with chronic liver disease and portal hypertension in both systemic and portal blood samples together with the histopathological scoring of liver biopsies.
Subjects: The control group 15 subjects free from any liver disease. The patient group 30 patients with chronic liver disease and schistosomal portal hypertension.
Methods: Clinical examination, abdominal ultrasonography, measurement of portal venous pressure and histopathological examination of liver biopsy. Laboratory investigations included evaluation of total nitric oxide (NO), endothelin-1 (Et-1) and type III procollagen (PIIINP) in both portal and systemic blood. In addition prothrombin, serum alanine, aspartate aminotransferase (AST, ALT), γ glutamyl aminotransferase (γGT) activities, serum bilirubin, albumin, serodetection of hepatitis B surface antigen (HBsAg) and hepatitis B core antibody and (anti-HCVAb).
Results:
- NO and ET-1 levels in both systemic and portal blood of SHF patients were significantly higher than in the control group.
- NO is a potent vasodilator.
- ET-1 increase may be a compensatory mechanism to antagonize the vasodilatory effect of NO.
- Child class B subgroup had higher NO and ET-1 than class A.
- NO and ET-1 levels did not differ between anti HCV positive and negative SHF patients.

PP-055 Development and application of a real-time TaqMan PCR for detection of the spotted fever group of rickettsia

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Objectives: Three species or subspecies of spotted fever group rickettsiae (SFRG) were documented in China. Recently, we detected a novel serotyping SFRG which was broadly prevalence in China. However, the greatest challenge in preventing and controlling rickettsiosis in China is laboratory diagnosis. Few clinical laboratories have the laboratory diagnostic ability for rickettisae and even some better equipped laboratories also used old Weil Felix. In order to solve this problem, our national laboratory for rickettsiosis surveillance has developed a Taqman real-time PCR assay based on the SFGR *ompA* gene that was devised for rapid detection of SFRG.
Methods: The primers and probes for the real-time PCR were designed based on the conserved sequences of the *ompA* gene through alignment from 14 SFGR species. Two hundred and sixty five blood samples (127 goats, 78 dogs, 60 cattle) collected from Yunnan Provinces were tested using the developed PCR to investigate the infection rate and the distribution among these animals.
Results: The developed assay amplified most of the SFGR strains: *R. sibirica, R. conorii, R. marmionii, R. rickettsii, R. africa, R. parkeri, R. canada, R. heilongjiangensis* but not *R. akari* and *R. felisi*. Genomic DNA from other members of the order Rickettsiales showed negative results. The mean (range) CV values for intra-assay and interassay variation were 1.15% and 2.59% respectively. The limits of detection (LOD) was 200 copies per reaction. Of the 265 animal blood samples, 56 (21.13%) tested positive. The prevalence among